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Serial No. 10/677,734

Customer No. 23379

Applicant: Gardner et al.

Confirmation No. 4912

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Group Art Unit: 1656

Docket No. UTSD:1510-1

Examiner: Swope, Sheridan

Title: *Foreign PAS Ligands Regulate PAS
Domain Function*

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DECLARATION UNDER 37CFR1.132

I, Professor Stephen R. Sprang, declare and state as follows:

1. I am a Professor in the Department of Biochemistry at the University of Texas Southwestern Medical School. The Board of Regents of the University of Texas System is the assignee of this patent application. I have authored numerous scientific papers in the field of protein regulation, and I am familiar with this patent application. A copy of my curriculum vitae is attached.

2. HIF2 α PAS B domain is an art-recognized, defined protein domain, and one skilled in the art does not require undue effort or experimentation to recognize and procure an HIF2 α PAS B domain for use in the claimed methods, as documented for example by Erbel *et al.*, *Proc. Natl. Acad. Sci.* 100(2003): 15504-9. In my opinion the Specification enables one skilled in the art to practice the invention without undue experimentation.

3. HIF2 α PAS B domain is an art-recognized, defined protein domain, and one skilled in the art has no trouble recognizing an HIF2 α PAS B domain for use in the claimed methods. There are many scientific publications describing the HIF2 α PAS B domain, and how to use it (e.g. Erbel *et al.*, 2003, *supra*). In my opinion the specification amply describes and exemplifies the claimed methods to one skilled in the art.

4. Vogtherr (2003) generally describes the use of NMR-based screening for lead discovery; Amezcua (2002) describes the use of NMR to detect ligand binding to PAS kinase; Tma (1997) reports that HIF1 α heterodimerizes with ARNT (note that HIF1 α is structurally and functionally distinct from the recited HIF2 α ; Sowter *et al.*, *Cancer Res.* 63(2003): 6130-4 and Raval *et al.*, *Mol Cell Biol* 25(2005): 5675-86); and Fukunaga (1995) reports identification of functional domains of the aryl hydrocarbon receptor.

Prior to the present disclosure, HIF was known to be regulated in several ways by oxygen availability, but only via mechanisms that are based on oxygen-sensitive enzymes that covalently modify portions of the HIF α subunit at sites distant to the PAS domains (Bruck & McKnight,

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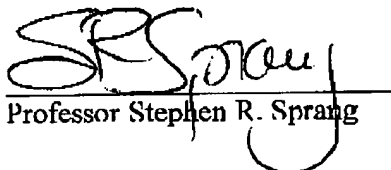
Science 294(2001): 1337-40; Jaakkola et al, *Science* 292(2001): 468-72; Ivan et al., *Science* 292(2001): 464-8; Lando et al., *Science* 295(2002): 858-61). These findings demonstrated two independent modes of oxygen regulation that do not involve the PAS domains, which taught away from any expectation that the HIF PAS domains would be sensory.

In addition, HIF2 α PASB presents a well-folded domain, which significantly contrasts with the dynamic regions of PASK PAS A (Amezcuca et al., *Structure* 10(2002): 1349-61; Erbel et al., 2003, supra), further removing any expectation of core ligand binding. Indeed, the structure of the ligand-free [apo] form of HIF2 α PASB is in contrast with the apo-structures of the many small ligand-binding protein domains, which either exhibit pre-formed cavities or pockets for ligands to bind or alternatively adopt an unfolded (and often, chaperone-bound) conformation. The HIF2 α PAS B structure shows neither of these.

Based on what was known prior to this disclosure, it is my opinion that one skilled in the art at the time of the filing date would not have expected HIF2 α PAS to provide a core for sensory ligand binding.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful, false statements may jeopardize the validity of the application and any patent issuing therefrom.

Date: June 19 2006


Professor Stephen R. Sprang